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In re Application of: Martinez et al. Art Unit: 1645

Application No. 10/009,660

Filed: December 7, 2001

For: METHODS AND COMPOSITIONS FOR OPSONOPHAGOCYTIC ASSAYS

Examiner: Hines, Jana A.

Date: August 28, 2003

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DECLARATION UNDER 37 C.F.R. § 1.132

1. I, Dr. George M. Carlone am a co-inventor named in the above-referenced patent application.

2. I have read and understand the above-referenced patent application, including the pending claims, and the Office action dated April 1, 2003.

3. The method disclosed in the above-referenced patent application has an important role in detecting functional antibodies to bacteria that undergo opsonophagocytic protection. The method allows one to simultaneously detect antibodies that recognize multiple bacterial serotypes. Previous methods for detecting antibodies that recognize a particular bacterial serotype, such as ELISA, only permitted detection of a single serotype at a time. Because previous methods only permitted detection of antibodies to one serotype at a time, these methods were both cost and labor intensive since large numbers of samples needed to be prepared and analyzed. In contrast, the method disclosed in the present application allows one to detect antibodies that recognize several different serotypes in a single sample, thereby reducing the amount of resources expended.

4. It is my understanding that in Paragraph 6 of the Office action of April 1, 2003, claims 11 and 21, which concern a method of determining the efficacy of an immunization, were rejected as not

sufficiently enabled by the specification. As shown in Exhibit A (Jodar *et al. Vaccine*, 21:3265-72, 2003), results obtained using the method disclosed in the present application correlate with ELISA results of serologic protection. The vaccine efficacy study used the PREVNAR vaccine, which is known to be effective when used in infants as part of their routine vaccination schedule.

5. Figure 1 of Jodar *et al.* shows the compiled ELISA results of 30,000 subjects for the seven serotypes present in PREVNAR. Each serotype was analyzed separately using ELISA, and the results compiled into a single figure. As shown in Figure 1, the PREVNAR vaccine is 97.9% efficacious, if the antibody concentration reaches a threshold of 0.20 μg/ml.

6. To demonstrate that there is a correlation between the efficacy results obtained with ELISA and functional opsonphagocytic activity, the method disclosed in the present application was used to analyze all seven serotypes for 79 of the 30,000 subjects represented in Figure 1. Figure 2 of Jodar *et al.* shows the result from one of the seven serotypes (four serotypes were analyzed simultaneously, but only one is shown in the figure for clarity). Similar results were obtained for the other serotypes. As shown in Figure 2, the method disclosed in the present application can be used to distinguish subjects who are protected by the vaccine, and those who are not. In addition, there is a correlation between the 0.2 μg/ml threshold antibody concentration obtained using ELISA (Figure 1) and the threshold opsonic antibody titer of 1:8 obtained using the opsonophagocytic assay disclosed in the present application (Figure 2). Therefore, the method disclosed in the present application, which detects functional antibodies to multiple serotypes simultaneously, was shown in Jodar *et al.* to indicate the efficacy of the PREVNAR vaccine.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

George M. Carlone
Dr. George M. Carlone

August 28, 2003
Date

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Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants☆

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Abstract

The World Health Organization (WHO) is undertaking a series of consultations on serological criteria for the evaluation and licensure of new formulations/combinations or different vaccination schedules of pneumococcal conjugate vaccines. The lack of a definitive serological correlate of protection and the multiplicity of antigens involved, especially since the clinical efficacy of most of the individual serotypes represented in the only licensed vaccine has not been established, are hindering the formulation of criteria for licensure of new formulations or combinations of the vaccine. This report analyses the various options with their relative merits and drawbacks and provides preliminary recommendations as guidance to regulatory agencies in evaluating these vaccines for the purposes of licensure. More detailed recommendations for production and control of pneumococcal conjugate vaccines, including criteria for evaluation for licensure, are currently being drafted.

Keywords: *Streptococcus pneumoniae*; Conjugate vaccine; Correlates of protection

1. Introduction

The World Health Organization (WHO) estimates that pneumococcal infections cause at least 1 million deaths annually worldwide [1]. Most of these deaths occur among young children in developing countries. A 7-valent pneumococcal conjugate vaccine was licensed in the United States in 2000 [2]. Licensure was based on a pivotal trial that established the efficacy of the vaccine against invasive pneumococcal disease among children in northern California [3]. However, this vaccine lacks a number of serotypes, such as 1 and 5, that are an important cause of invasive pneumococcal disease in South America, Africa and Asia [4]. Newer formulations containing 9 or 11 serotypes are currently under development [5–7].

A variety of formulations and presentations of 7-, 9- or 11-valent pneumococcal conjugate vaccines, either as stand-alone products or in combination with other antigens such as *Haemophilus influenzae* type b (Hib) or *Neisseria meningitides*, may be required to accommodate the needs of individual countries or regions. Additional differences in formulations required in developing countries include combinations containing diphtheria–tetanus–whole cell pertussis (DTwP), and multi-dose formulations containing thiomersal. Furthermore, differences in the epidemiology of pneumococcal disease may require modifications of the immunization schedule in certain regions.

☆ Use of vaccine trade names in this document is for identification only and does not imply endorsement by the authors or their institutions.

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EXHIBIT A

For the purpose of licensure, non-inferiority f the newer formulations to the existing licensed formulation will have to be established. The multiplicity of antigens in these formulations will make such an assessment very complex. Establishing non-inferiority against clinical trial end-points for each new formulation is impractical. At the same time, establishing non-inferiority based on serological criteria is rendered difficult by the fact that there is no clear agreement on the concentration of antibody or titre in vaccinees that may be used in a non-inferiority model to predict whether a vaccine is effective. The lack of such data may result in efficacious vaccines being rejected on the basis of arbitrarily determined antibody concentrations. This could result in the production of vaccine being dependent on a single manufacturer, which could compromise global vaccine supply. In addition, this may also limit the number and types of combinations and formulations available for use, which would be detrimental from a public health point of view.

Because of possible differences in the optimal formulation, serotype distribution, disease burden and epidemiology, clinical trials may still be necessary in developing countries. These trials may provide additional serotype-specific efficacy. These data will help reinforce and refine the use of serologic end-points and the strategies outlined in these recommendations.

In order to discuss these issues and provide guidance to regulatory agencies for evaluation of new pneumococcal conjugate vaccine formulations based on serological criteria, WHO sponsored a consultation in Anchorage, Alaska in May 2002. The consultation reviewed available data on serological criteria that predict protection against pneumococcal disease and formulated a series of recommendations that may serve as important elements for the WHO recommendations for production and quality control of pneumococcal conjugate vaccines. This report describes the major scientific challenges for establishing a licensing pathway based on serological criteria and summarises recommendations made at this meeting.

2. General considerations for licensing pneumococcal conjugate vaccines

National control authorities are risk averse. Therefore, they expect data that provide high certainty that a new product is both safe and efficacious before approving it for licensure. While data establishing clinical protection are optimal, it is recognised that such data cannot always be obtained. Consequently, vaccines have been licensed purely on the basis of immunogenicity data, provided the criteria used predict clinical protection with a high degree of certainty. This pathway has been used for licensure of Hib and group C meningococcal conjugate vaccines.

The United States Food and Drug Administration (USFDA) has approved three different Hib conjugate vaccines for licensure. In 1990, it approved the first two, HibTITER™ (Wyeth Vaccines, Pearl River, New Y rk) and PedvaxHIB™ (Merck, West Point, Pennsylvania) based on phase III clinical efficacy data [8,9]. In 1993 the third one, ActHIB™ (Aventis Pasteur, Lyon, France), was approved for licensure following evaluation of immunological data based on the criteria outlined below [10]:

(a) assessment of antibody responses, as measured by ELISA, in randomised comparative immunogenicity studies in infants with currently licensed vaccines as the control;
(b) persistence of antibodies after the primary immunisation series until the recommended booster dose is given;
(c) demonstration that the conjugate vaccine primes infants for a subsequent booster response to the native Hib polysaccharide, indicating the induction of immunologic memory;
(d) demonstration of functional capacity of conjugate vaccine-induced antibodies (e.g. measured either by opsonic or bactericidal activity).

The primary end-point for establishing non-inferiority was the proportion of vaccinees who developed antibody above a threshold concentration of 1.0 μg/ml, although it has been argued that maintaining anti-PRP concentrations of ≥0.15 μg/ml correlates better with protective efficacy [11].

Similarly, group C meningococcal conjugate vaccines were first licensed in the United Kingdom in 1999, based on their immunogenicity rather than clinical efficacy. The antibody data supporting the use of group C meningococcal conjugate vaccines in the United Kingdom, were generated by serum bactericidal assay (SBA) using rabbit complement. There is a general consensus that when baby rabbit serum is used as the source of complement, SBA titres of <1:8 are predictive of susceptibility to invasive meningococcal disease and titres of ≥1:8 are predictive of short-term protection. In the UK, a combination of additional indicators was used to assess immune response to license a meningococcal group C conjugate vaccine. These include: (a) evidence of a four-fold rise in antibody titre between pre- and post-immunisations sera; (b) demonstration of immunological memory; or (c) evidence of increased avidity of group C-specific antibody [12,13].

While a similar strategy could be adopted for pneumococcal conjugate vaccines, the issue is complicated by the fact that unlike Hib and group C meningococcal conjugate vaccines, this vaccine is a mixture of multiple protein-conjugated polysaccharide antigens. The fact that newer formulations may contain additional antigens for which no licensed product is available for comparisons adds complexity to the registration pathway.

On a more pragmatic side, one would need to reconcile the importance of defining immune response measurements that reliably predict effectiveness with the importance of access to additional pneumococcal conjugate vaccines. A first major consideration deals with the availability of other pneumococcal conjugate vaccines in the short- and mid-term

apart from the already licensed Prevnar™. Balancing factors that would make a vaccine acceptable for licensure even if 'inferior' in one or more aspects f comparison with the licensed vaccine need to be considered. When in March 2001, the USFDA Vaccines and Related Biological Products Advisory Committee (VRBPAC) was asked for advice regarding whether non-inferiority immune response trials comparing a new pneumococcal conjugate vaccine containing seven or more serotypes with the licensed one would be sufficient for inferring efficacy against invasive pneumococcal disease, the majority of the committee agreed that non-inferiority would be sufficient. However, inferiority for rare serotypes might not be as important relative to the benefits of either having additional vaccines licensed or additional serotypes covered.

3. Serologic methods for defining immunological correlates of protection

The serologic methods used to evaluate the licensed 7-valent pneumococcal conjugate vaccine included two "critical" tests, i.e. IgG quantitation of all specimens and opsonophagocytic assays on a subset of specimens. Additional tests, i.e. isotype/subclass, avidity, and serotype cross-reactivity, have provided qualitative information about immune mechanisms evoked by this vaccine [14,15]. However, interpretation of the results of multiple tests performed on one specimen can be challenging.

The potential issue related to the use of these tests is the reported modest relationship between the ELISA IgG concentrations and opsonophagocytic titres [16]. This appears to be a greater problem with pre-immunization sera derived from vaccine naive adults than with post conjugate vaccine sera from infants or toddlers. Several factors, including the quality of the assays and the sera being analysed, may contribute to the modest correlation. The accuracy of the ELISA may be influenced by the substances in the sera, the quality of the reagents/materials and steps used in the assay. The outcome of the opsonophagocytic assays are affected by the type of phagocytes, bacteria and complement used in the assay. In addition, titres and therefore correlation between these assays, may be affected by characteristics of the population from which the sera is obtained or the presence of pre-existing antibodies or other pneumococcal antigens in the sera. Optimization of the assays has improved the correlation between ELISA and the opsonophagocytic assays [17,18]. IgG antibody concentrations as measured by ELISA appears to be the best parameter to use as the primary criteria for licensure of new formulations for the following reasons: (1) IgG is the desired immune response; (2) the methodology for measuring it is validated in infants; (3) a bridge to efficacy data has been established; and (4) a cross-laboratory standardization process has been completed. The functional opsonophagocytic assays will provide critical supplementary data for the serotypes included in Prevnar™ and primary data on additional serotypes in new vaccines. Other serologic tests can provide additional descriptive data but have not yet been standardised and have not proven to be predictive of protective efficacy.

Finally, there are also likely to be statistical challenges with the use of serological criteria for licensure of pneumococcal conjugate vaccines. Newer formulations with nine or more different immunogens will be subject even to bigger problems associated with multiple statistical comparisons than the currently licensed 7-valent pneumococcal conjugate vaccine. Antibody responses to some components of the new vaccine may be higher or lower than for the licensed product, further complicating the analysis.

3.1. Serological predictors of protection against invasive disease inferred from pneumococcal conjugate vaccine efficacy trials

The serological criteria that may predict protective efficacy against invasive pneumococcal disease are not precise concentrations but rather estimates or threshold levels that predict protection. The estimates are based on efficacy data that are themselves not very precise, i.e. have wide confidence limits. Moreover, the serological criteria used for encapsulated bacteria are often from antibody binding assays, e.g. ELISA or RIA, which are surrogate (correlative) measurements for the likely protective activity, i.e. bactericidal or opsonic antibody.

Serological criteria for evaluation of pneumococcal conjugate vaccines will be easier if serotype-specific efficacy data were available. However, in the Kaiser–Permanente trial, serotype-specific efficacy was shown for only four of the seven serotypes included in the vaccine. Licensure was based on aggregate efficacy, understanding that immune responses and efficacy may vary between serotypes. In the absence of serotype-specific efficacy data against invasive pneumococcal disease, it will not be possible to define specific serological criteria for each serotype contained in the vaccine.

In order to establish a threshold concentration of antibody that predicts protection two major simplifying assumptions may be made. The first assumption is that antibody concentration after the primary series of three doses of vaccine predicts protection. Since there is conclusive evidence of aggregate efficacy after a primary series of three doses as well as after four doses, it is reasonable to use serological measurements after either three or four doses as criteria for evaluation. Post dose three assessment is more stringent because the antibody concentrations and functional activity are lower than after the fourth dose and the highest age-specific disease risk is between the third and fourth doses. Moreover, in many countries a three-dose schedule is more acceptable than a four-dose one. The second assumption is that the relationship between risk of disease and antibody concentration is expressed in a step-wise rather than in a continuous function, whereas in reality the relationship is continuous.

Taking into consideration these two assumptions, there are two options for estimating the antibody threshold that predicts protection against invasive disease: (a) protective concentrations are relatively similar for all types and that one level may be used for all types; and (b) concentrations are different for each serotype.

Since the vaccine efficacy (VE) for invasive disease is known one may apply the following relationship to define the antibody concentration that predicts protection:

$$\mathrm{VE} = 1 - \frac{\text{probability of disease in Vax group}}{\text{probability of disease in control group}}$$

$$\mathrm{VE} = 1 - \frac{\%\text{ of Vax subjects with [Ab]} < \mathrm{Ab}_{\mathrm{protective}}}{\%\text{ of control subjects with [Ab]} < \mathrm{Ab}_{\mathrm{protective}}}$$

If one applies this relationship to the aggregate VE data from the Kaiser–Permanente efficacy trial [2], and if the antibody concentrations in the control population are ignored, a serotype non-specific threshold concentration (i.e. option (a)) of 0.20 μg/ml is obtained (see Fig. 1).

If one uses option (b), an antibody threshold can only be only be derived for serotype 19F using the same formula and this is 0.4 μg/ml. For the other three serotypes for which efficacy data are available, a threshold cannot be defined using the reverse cumulative distribution curves, because no vaccine failures were observed.

In addition to the above analysis, there are several additional factors that support the use of the 0.20 μg/ml threshold for predicting protection against invasive disease.

1. An antibody concentration ≥0.20 μg/ml corresponds to the threshold of opsonic antibody titre of 1:8 (Fig. 2).
2. This threshold concentration also appears to predict the age-specific disease rates, i.e. the rates increase when passively acquired antibody concentrations decline below ≥0.20 μg/ml and then decrease again when naturally acquired antibody concentrations increase above this concentration (Fig. 3).

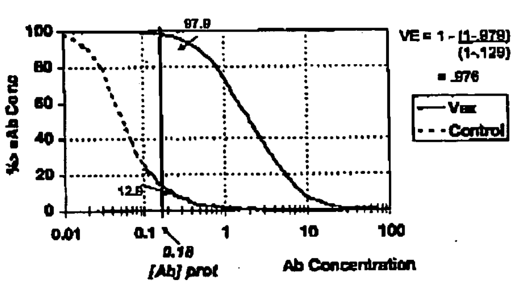

Fig. 1. Reverse cumulative distribution curves of antibody concentrations post dose 3 in vaccine and control groups. Data source: [3]. The reverse cumulative distribution curves were based on the pooled Ab concentrations of the seven serotypes.

Table 1
Correlation between post dose 3 and post dose 4 ELISA antibody (D118-P16)

Serotypes	Pearson correlation[a] $N = 269$–271	P-value
4	0.441	<0.001
6B	0.526	<0.001
9V	0.439	<0.001
14	0.341	<0.001
18C	0.498	<0.001
19F	0.306	<0.001
23F	0.545	<0.001

[a] Correlation based on post dose 3 and post dose 4 Ab concentrations on log-scale.

3. This threshold is consistent with available data from passive immunization using bacterial polysaccharide immune globulin (BPIG) to prevent pneumococcal otitis media [19] and invasive pneumococcal disease [20].
4. This threshold appears to clearly discriminate between conjugate vaccinees and controls in immunogenicity studies (Fig. 1).
5. Infants with antibody ≥0.20 μg/ml after conjugate vaccine show evidence for priming for a subsequent response to the capsular polysaccharide (Fig. 4). Indeed, even immunized children who failed to reach the 0.2 μg/ml threshold showed evidence of priming.
6. Post dose three-antibody response is correlated with booster response to conjugate and polysaccharide (Table 1).

Relatively modest differences in the point estimate of efficacy may significantly influence the threshold antibody concentration derived by the method suggested above. This underlines the importance of precision of the efficacy estimate in deriving threshold antibody concentrations that predict protection. Pooling efficacy data from all the completed efficacy trials may provide higher precision for the efficacy estimates and more precise information on the serological criteria. Moreover, pooling data would mean that different ethnic population would be represented in the data making it more widely applicable.

While it is clear that a threshold concentration that predicts protection may be different at least for a few serotypes, the absence of precise efficacy for many of the serotypes make type-specific threshold difficult to define. Moreover, it is unlikely that type-specific thresholds could be defined for additional serotypes in formulations that had not undergone efficacy trials. Since the only licensed formulation was approved on the basis of aggregate efficacy, the same principle may be used with serological criteria, i.e. use a common threshold for all serotypes assuming that the concentration is relatively close for most serotypes.

The currently licensed vaccine was licensed based on efficacy against bacteraemia and meningitis. Serological criteria that predict protection against this end-point may be derived from the efficacy and immunogenicity data. However, these

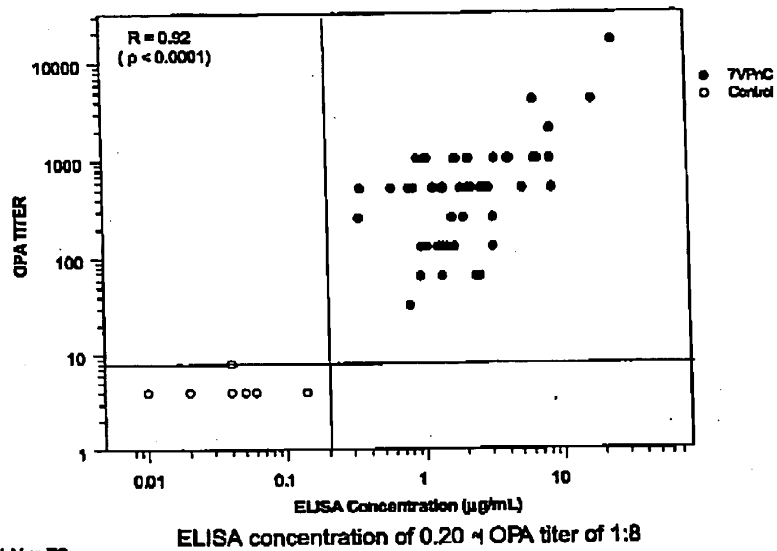

Fig. 2. Comparison of post dose 3 opsonophagocytic (OPA) titres and ELISA antibody concentrations for S. pneumoniae serotype 4 (types 6B, 9V, 14, 18C and 23F are similar). Data source: Lederle Laboratories. Data on file: Manufacturing bridging study of 7-valent pneumococcal conjugate vaccine, D118-P16 (France et al.).

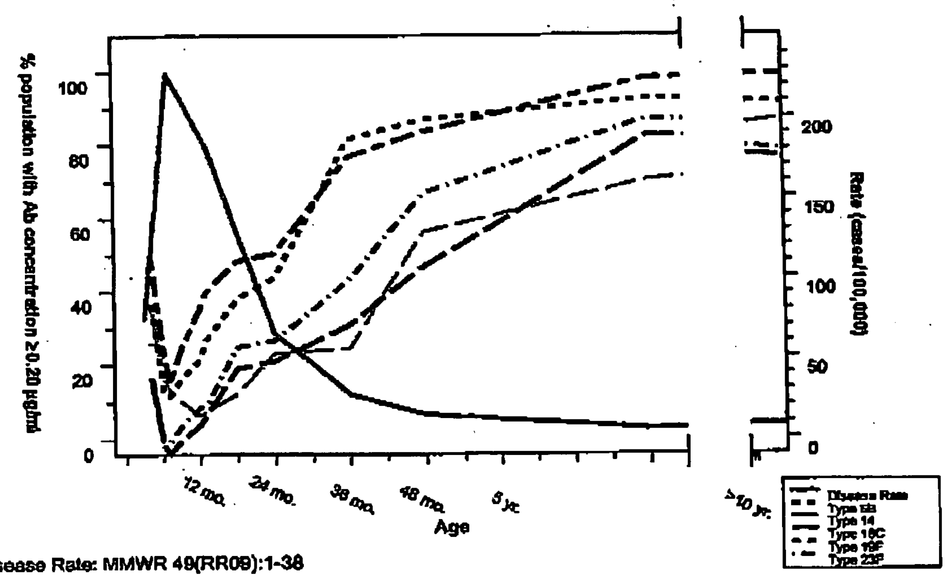

Fig. 3. Age-specific invasive disease rates and proportions with antibody concentration ≥ 0.20 mcg/mL. Data source: antibody concentrations: [3,30,31]. Lederle Laboratories. Data on file: Safety, tolerability, and immunogenicity of 7-valent pneumococcal CRM_{197} vaccine in children between 1 and 9 years of age, D118-P18 (Black et al.). Lederle Laboratories. Data on file: Safety and immunogenicity of a single injection of pneumococcal CRM_{197} vaccines in healthy adults, D124-P1 (Steinhoff et al.).

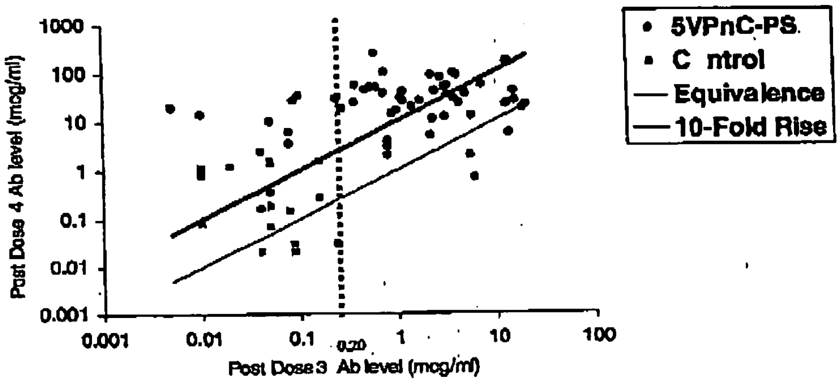

Fig. 4. Comparison of antibody concentrations against serotype 6B post dose 3 and post dose 4. Data source: Lederle Laboratories. Data on file: Immunogenicity of a booster dose of 23-valent pneumococcal polysaccharide vaccine in children who were previously immunized with three doses of pneumococcal conjugate vaccine, D92-P5 [32].

criteria may not predict protection against the more common diseases caused by the pneumococcus, namely pneumonia and otitis. Currently available efficacy data may not be sufficient to derive criteria specific for these end-points. It is unlikely that even in the ongoing or recently completed trials serotype-specific efficacy will become available for pneumonia. Hence, licensure of future formulations will have to be based on invasive disease end-points.

Other options for establishing serological criteria that predict protection are the use of geometric mean concentration (GMC) of antibody and seroconversion rates. While GMC may be better to predict mucosal protection, herd immunity, and efficacy against vaccine-related types, proportion of responders reaching a threshold level may be best predictor of protection against invasive disease. The lack of correlation between serotype-specific GMCs and serotype-specific protection against acute otitis media (AOM) suggests that GMC as such may not be an appropriate end-point even for prediction of immunity to mucosal disease [21]. The antibody concentration needed for protection against AOM seem to differ by serotype. This has been shown by models which have included serotype-specific antibody concentrations and the risk of acute otitis media caused by the serotype among vaccinated children [22]. Seroconversion could be used as a criterion for serotypes for which efficacy data were not available. However, seroconversion will be difficult to define in the presence of passively acquired maternal antibody.

4. The role of immunological memory in protection against pneumococcal invasive disease

There is now evidence to show that conjugate vaccines are T-cell dependent antigens and that they induce immunological memory in the form of an expanded pool of memory B cells [14,23]. Antibody concentrations may gradually diminish after a primary series of doses and these concentrations may or may not fall below the estimated protective threshold. It is expected that subsequent natural exposure to the pathogen or another dose of the vaccine will elicit a booster response and result in a large increase in antibody production so that the antibody concentrations may exceed the protective threshold concentration within 5–7 days of exposure. It is important to note that immunological responses may differ between the licensed and new vaccine depending on when the serum was collected relative to the administration of the priming series, any subsequent booster dose or natural immunizing exposure to the organism.

Existing data suggest that circulating antibody, induced by the primary series of vaccination with Prevnar™, was maintained as a result of priming [24]. Evidence that pneumococcal conjugates induce immunological memory raises the question of whether memory alone is sufficient to confer protection and whether it will only be sufficient to offer protection against invasive disease or whether it will also provide protection against AOM. Studies on the natural history of disease and acquisition of immunity in infants suggest that the failure to mount a satisfactory immune response to capsular polysaccharide leaves them more prone to pneumococcal infection. Over time, carriage of the organism repeatedly maintains natural immunity offering protection throughout most of the remainder of an individual's life. Only in old age, as a consequence of waning immunity and other non-immunological factors, pneumococcal disease once again becomes an important cause of morbidity and mortality [25,26]. On this basis, it appears to be reasonable to predict that induction of immunological memory will be sufficient for long-term protection against disease.

For the purposes of vaccine evaluation and licensure it is important to agree upon a common definition of immunolog-

ical memory to a polysaccharide antigen. It can be defined as a response that is (a) present in an therwise non-responsive individual (e.g. infants who do not normally respond to certain polysaccharide antigens), (b) characterised by a higher antibody response that is dominated by IgG on exposure to an antigen, and (c) characterized by antibodies with increased affinity or avidity as a result of affinity maturation. Thus, possible approaches to measuring memory could include: (a) evidence of a boostable/augmented immune response with either polysaccharide or conjugated antigen; (b) the presence of a response dominated by IgG; and (c) increased antibody avidity. Generally, there is a relationship between antibody avidity and its functionality, in that higher avidity antibodies are functional at lower concentrations than lower avidity antibodies [27]. However, antibody concentration does not always predict antibody avidity. Evidence suggests that a single dose of conjugate vaccine may be sufficient to increase antibody avidity [28,29], that higher avidity antibodies were more cross-reactive with closely related pneumococcal serotypes [28,29], and that memory probably can be predicted from the primary response (Table 1), although priming can occur in the absence of active antibody production (Fig. 4). For the purposes of vaccine evaluation, the simplest method of demonstrating memory would be the increased concentration and IgG dominance of an antibody response following a booster dose.

5. Conclusions

Based on the discussions and deliberations at the meeting, the following conclusions were drawn:

5.1. Primary end-point

- IgG antibody concentrations as measured by ELISA 4 weeks after a three-dose priming series would be the optimal primary end-point and main licensing parameter.
- A single threshold antibody concentration that predicts protection against invasive disease should be used for all pneumococcal serotypes. This threshold will be determined through an analysis that pools data from the efficacy trials with invasive disease end-points that have been completed to date.
- The percentage of responders (to be determined following the definition of the threshold concentration) should be used as the criteria to determine non-inferiority.
- A single primary end-point is sufficient for registration.

5.2. Secondary end-points to support licensure

5.2.1. Functional antibodies

- Opsonophagocytic activity as measured by opsonophagocytic assay after a three-dose priming series is required to demonstrate the functi nality of antibodies; the opsonophag cytic activity should be compared ideally to an age-matched non-immunized population, as antibodies to other antigens can demonstrate opsonophagocytic activity.
- Cross-laboratory standardization of OPA should be conducted as soon as possible, and WHO would take appropriate steps to accelerate this process.

5.2.2. Immunological memory

- Evidence of memory will be shown by administration of a booster dose of pneumococcal PS vaccine and comparison of concentrations between age matched unprimed and primed individuals.
- At this stage, use of a fractional dose of PS as booster is only a possibility but not sufficiently tested.
- Avidity of antibodies is also a useful marker for immunological memory.

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